Interactions of Organophosphorus and Related Compounds with Acetylcholinesterase

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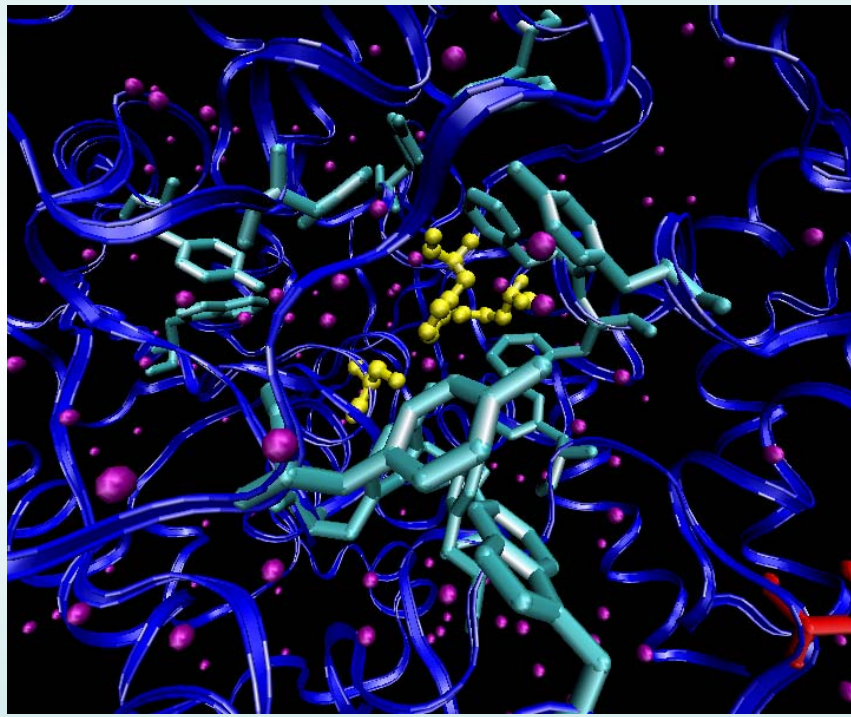
Technical Goals

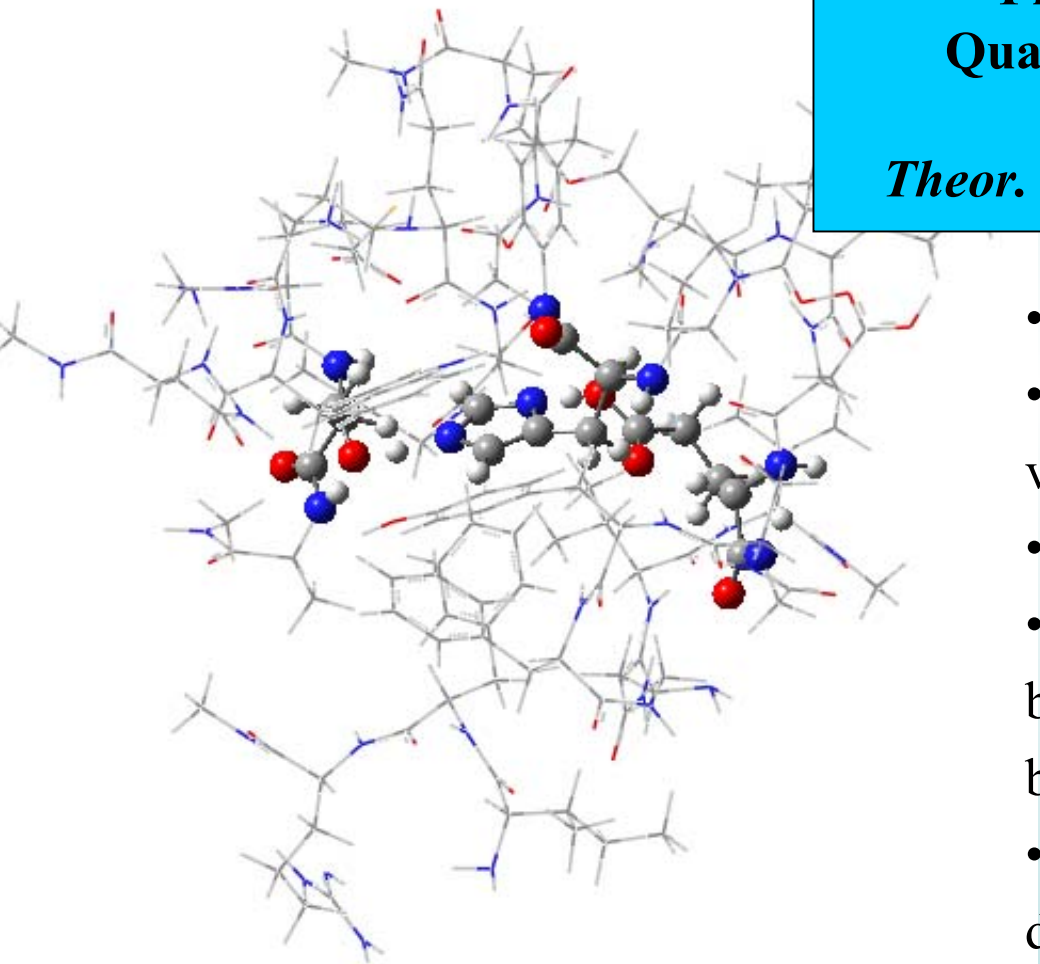
Objectives: **Understanding of :**

- **reversible and irreversible binding by OP and other compounds in active site of acetylcholinesterase**
- **role of solvent**
- **mechanism of oxime therapy**
- **'Aging' mechanism**

by theoretical modeling to facilitate development of new compounds for therapeutics and prophylaxis.



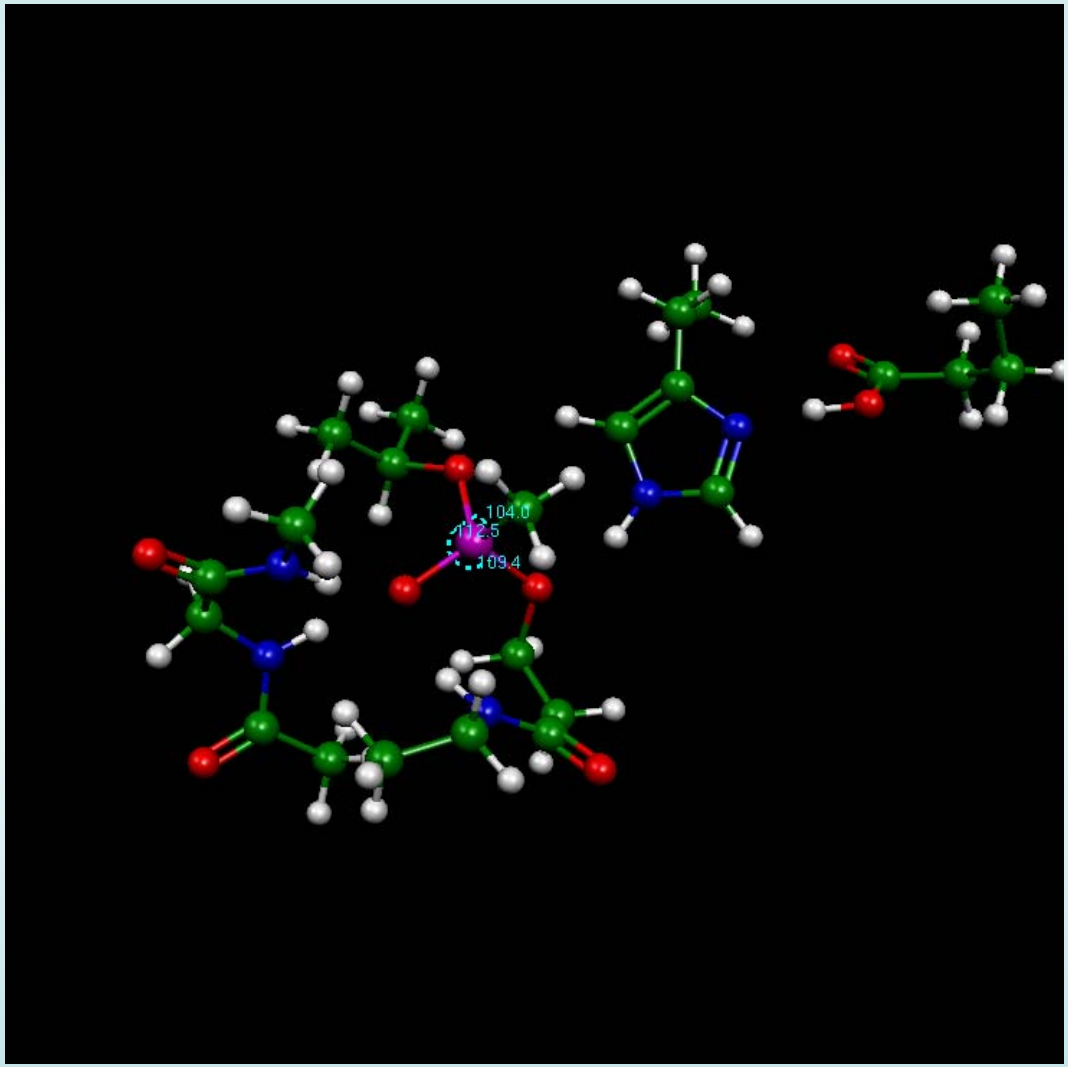
Previous Results



AChE:
Previous results used Mixed Quantum/Classical and QM/QM Modeling
***Theor. Chem. Acc.* (2003) 1009:160-168**

- Excellent alignment of catalytic triad
- Some sensitivity to algorithm (ONIOM vs SIMOMM), forcefield, model size
- ~15 kcal/mol TS barrier for OP binding
- QM/QM shows charge transfer between OP and oxyanion hole on binding
- Starting geometries taken from exptl data

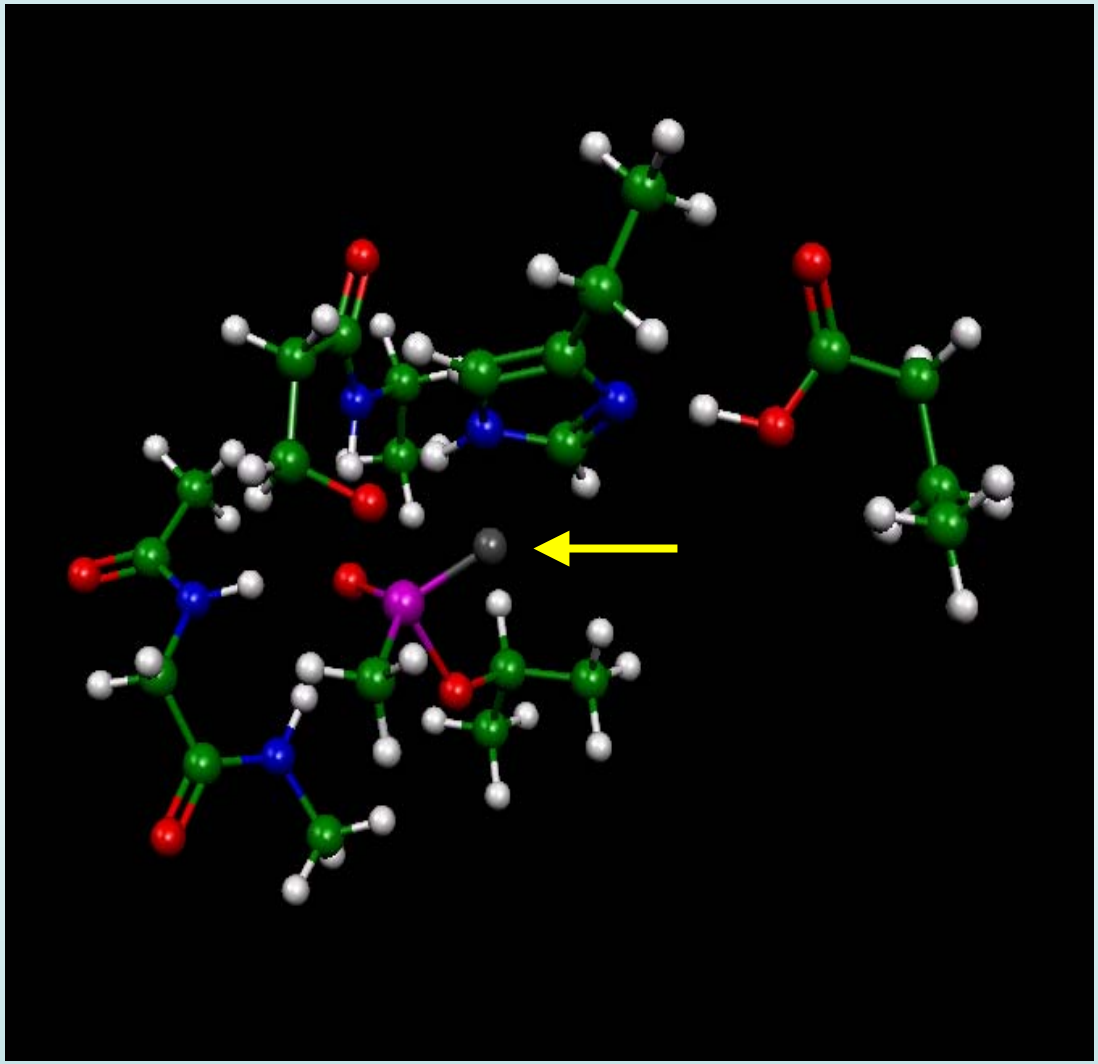
Organophosphate Family



B3LYP/3-21G

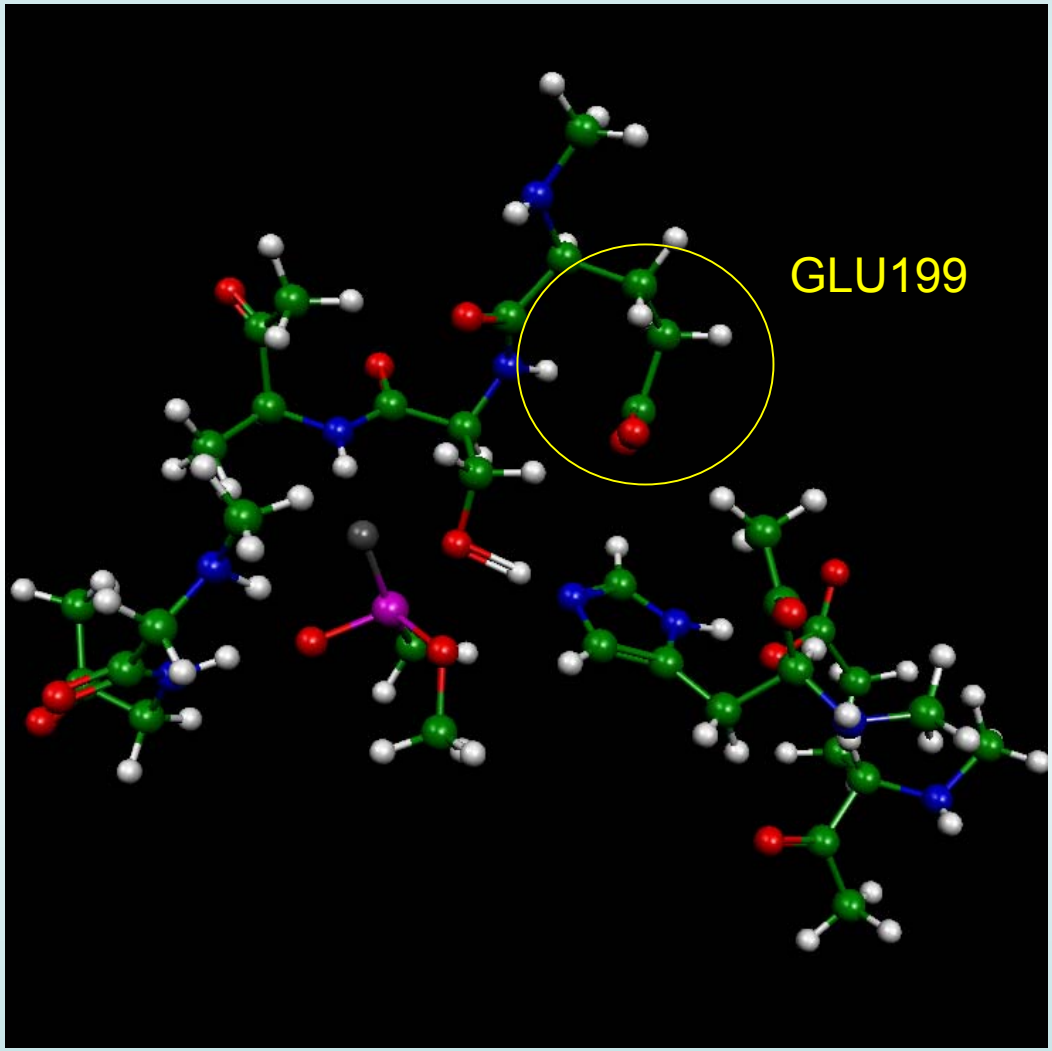
- Maintain strong bonding within catalytic triad
- Maintain H-bonding with Oxyanion Hole
- Tetrahedral intermediate without F- (LG)
- VERY Stable trig. bipyr. with F- (LG)
- Sarin, Soman, Tabun, DMMP, DFP, DEFP

Orientation and Structure



- Apical leaving group preferred.
- May bind with equatorial leaving group, product higher in energy by 10+ kcal/mol
- Multiple minima WRT sidechain orientation, within ~3 kcal/mol of each other

Large Enough Yet?

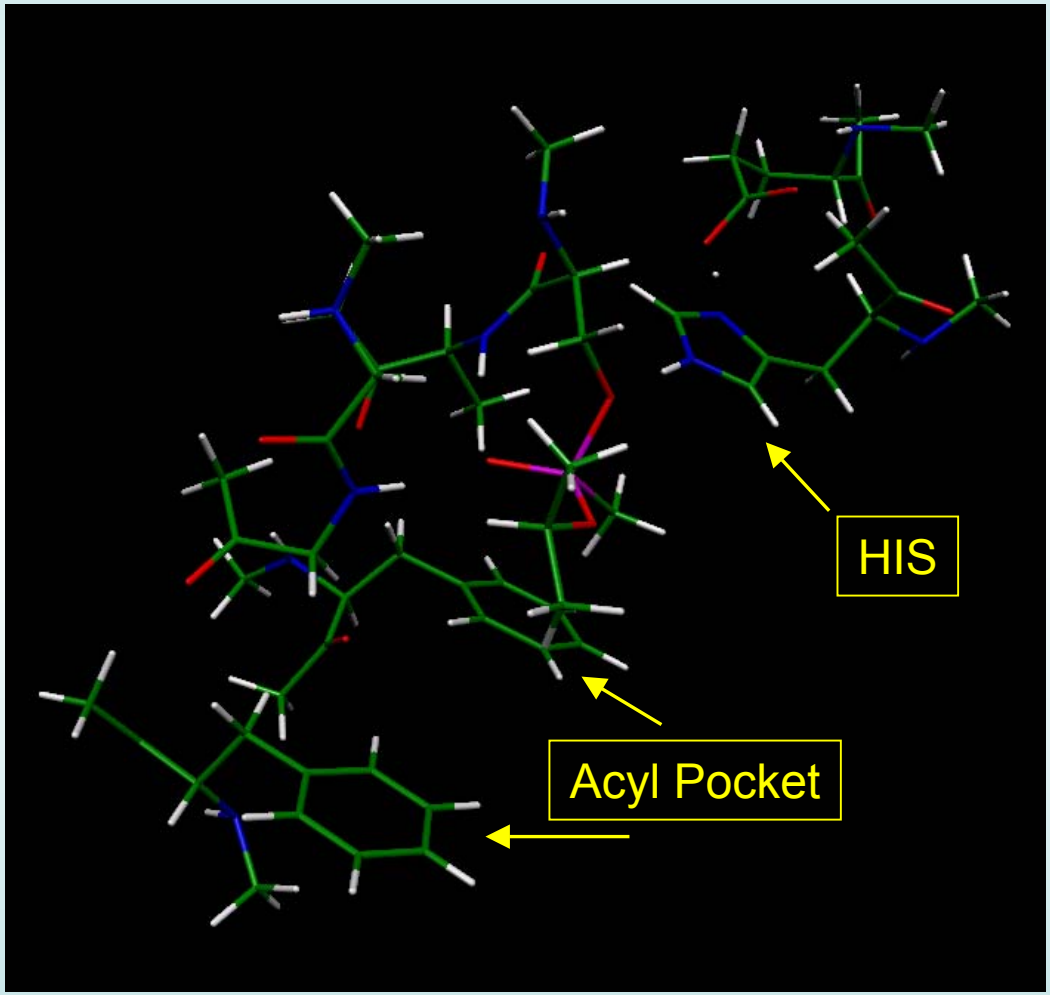


B3LYP/3-21G

- Note GLU199 swinging over HIS to stabilize
- ***Additional*** H-bond to HIS and not ***alternative***, as per Kovach et al.
- Bonding to oxyanion hole (OX) maintained
- LG intact, alternative H-bond w/OX

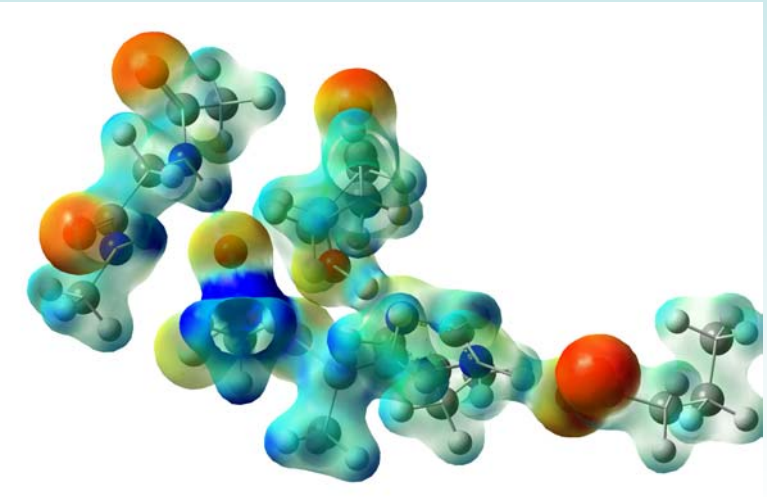
Still Larger?

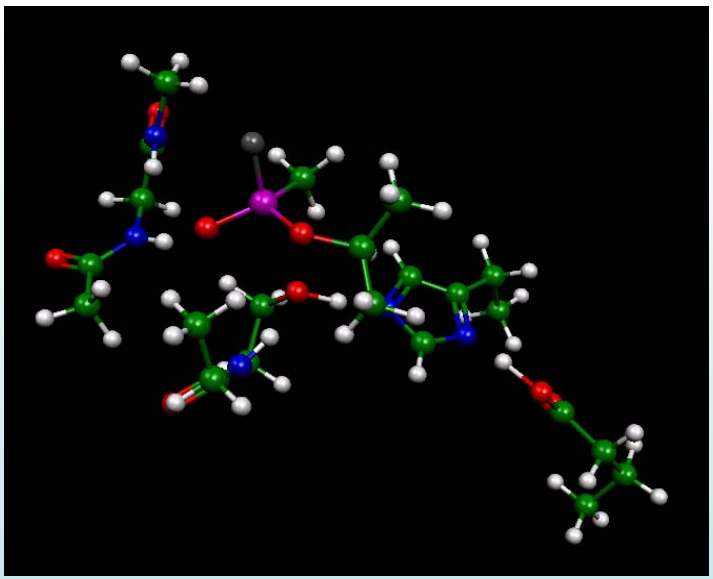
B3LYP/3-21G



- Acyl Pocket (AP) contribution minimal in
  - reactant
  - product
  - product with LG gone
  - aged product
- No sign of affect on stereoselectivity
- Minimal steric hindrance for iso-propoxy sidechain
- Look at bulkier systems?

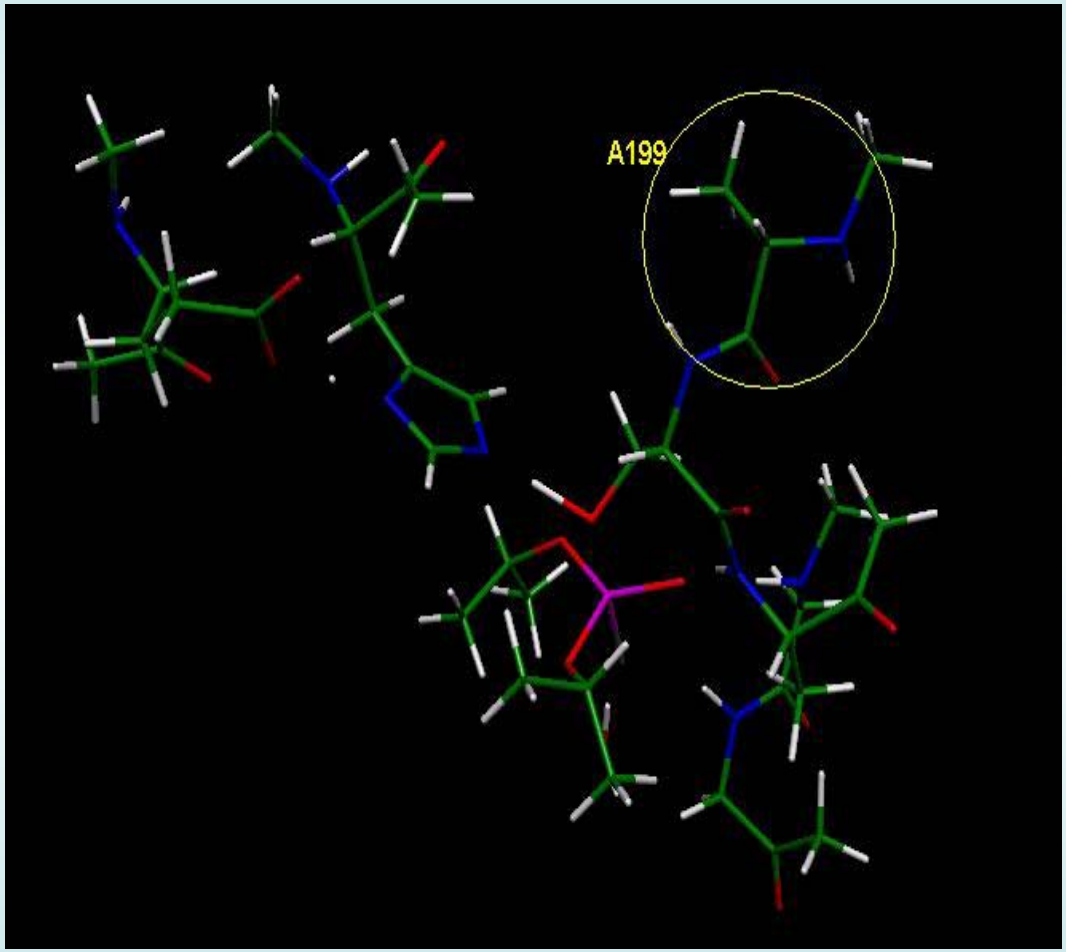
Chirality





- S product uniformly lower in energy for a variety of OP compounds (varying alkyl sidechains)
- R sarin bound product not isolated
- R reactant (unbound) complex ~16 kcal/mol higher in energy than S sarin (bound) product
- Energy difference between enantiomers found even in truncated model (Act + OX)
- P-O orientation into Oxyanion Hole maintained

Mutagenesis



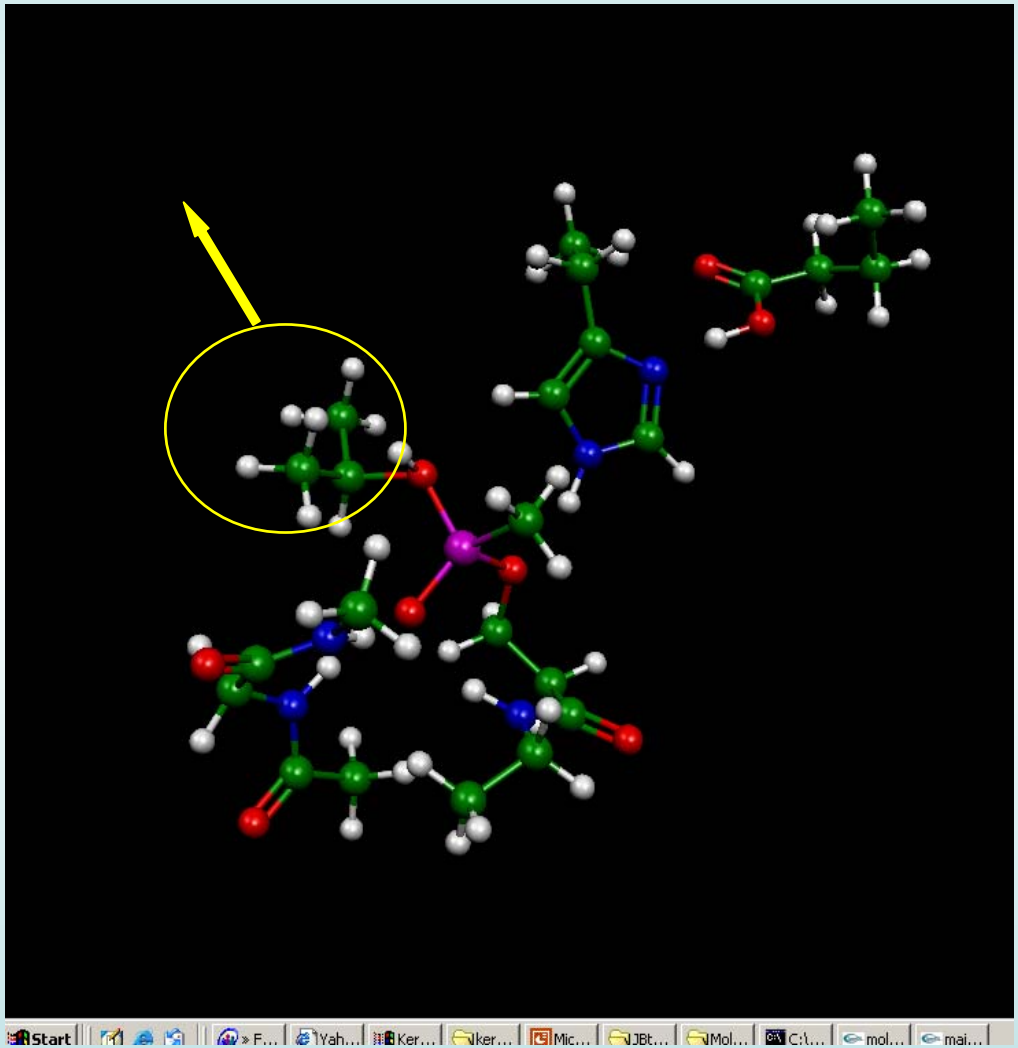
$$E + PX \rightleftharpoons EPX \longrightarrow EP+X$$

$$\xrightarrow{k_i}$$

Exptl evidence of ~15-fold decrease in DFP k_i with E202A mutation in HuAChE (Ordentlich et al JBC **271** 11953 (1996))

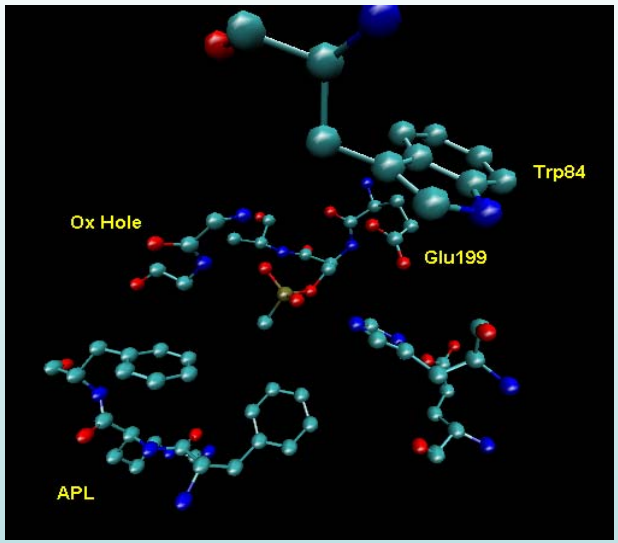
EPX complex state for DFP not isolated, energetics underway.
Repeat for DEFP, paraoxon

Aging

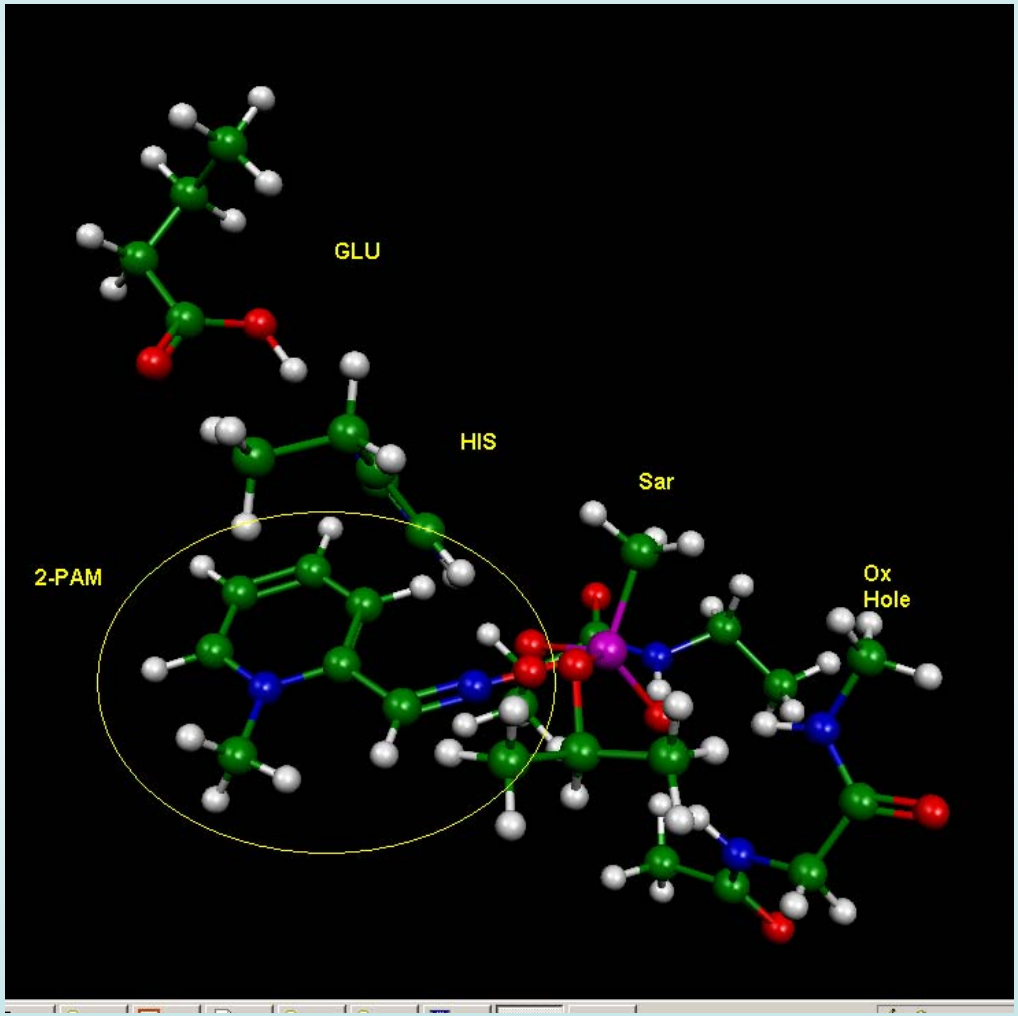


B3LYP/3-21G

- Mechanism-transient carbocation vs. push-pull
- Glu199,W84?
- Mapped out high barrier to carbocation mechanism (~40 kcal/mol) w/o proton, repeating with proton

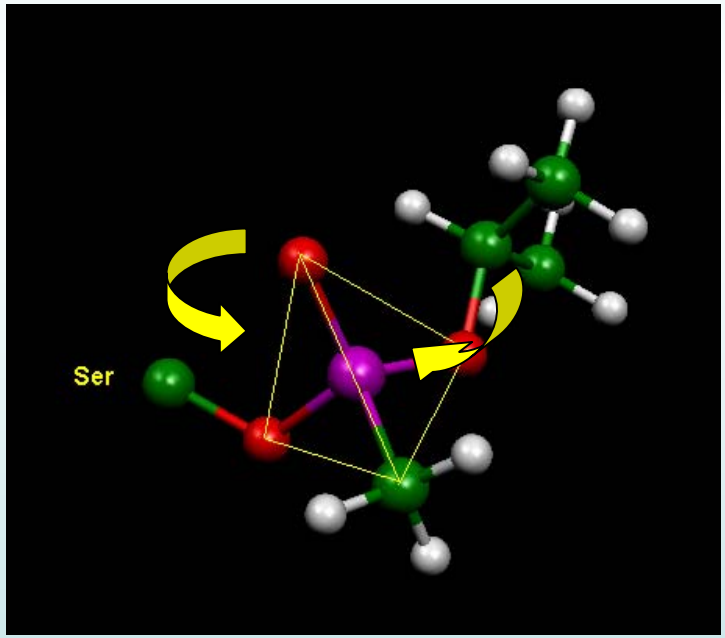


Reactivators - 2PAM



Sensitivity to face of attack?

Berman *Structure and Function of Cholinesterases and Related Proteins* ed. Doctor *et al.,* Plenum Press, New York, 1998, pp. 413-417.

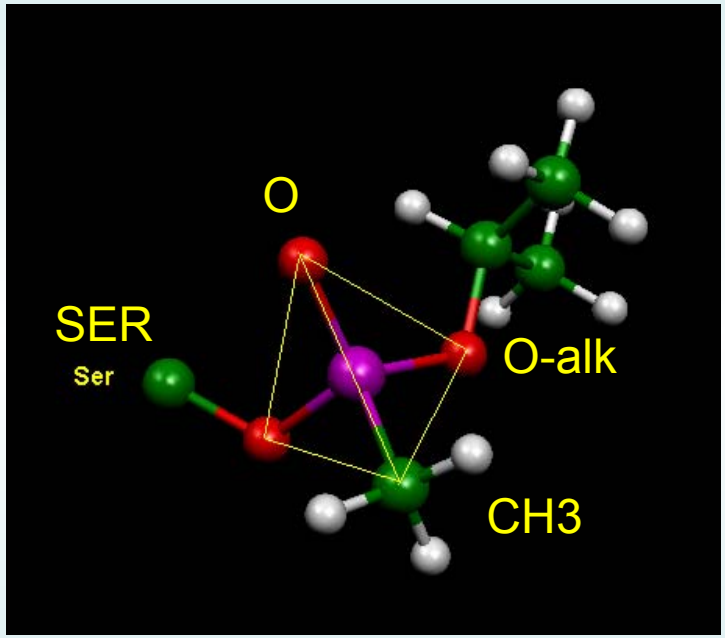


Reactivators - 2PAM

- SER-Oalk-O face X
- CH3-Oalk-SER face X
- O-Oalk-CH3 face X
- O-Oalk-CH3 face with H2O X

Result in –OX in apical position

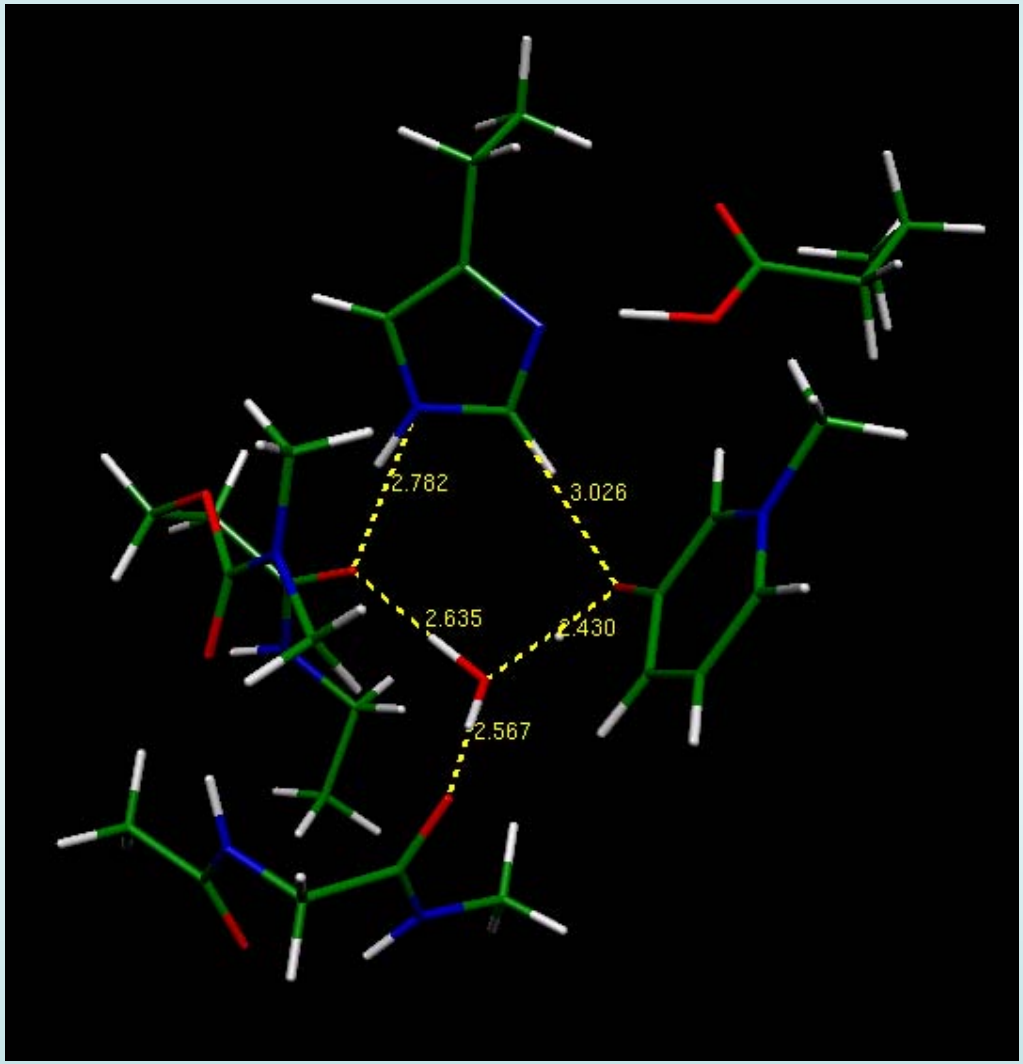
- Steric hindrance at edge

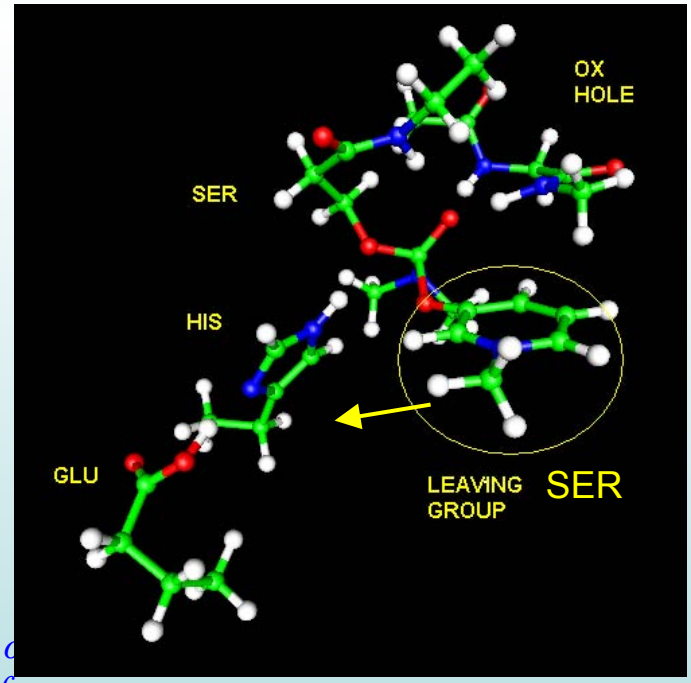


I have not failed, I have only discovered 10,000 ways that do not work. –T.A. Edison

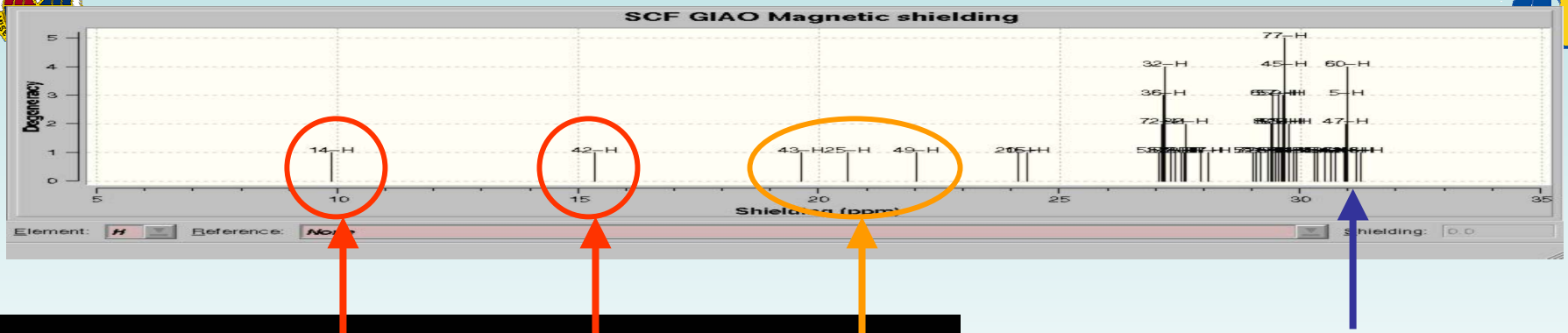
Prophylaxis

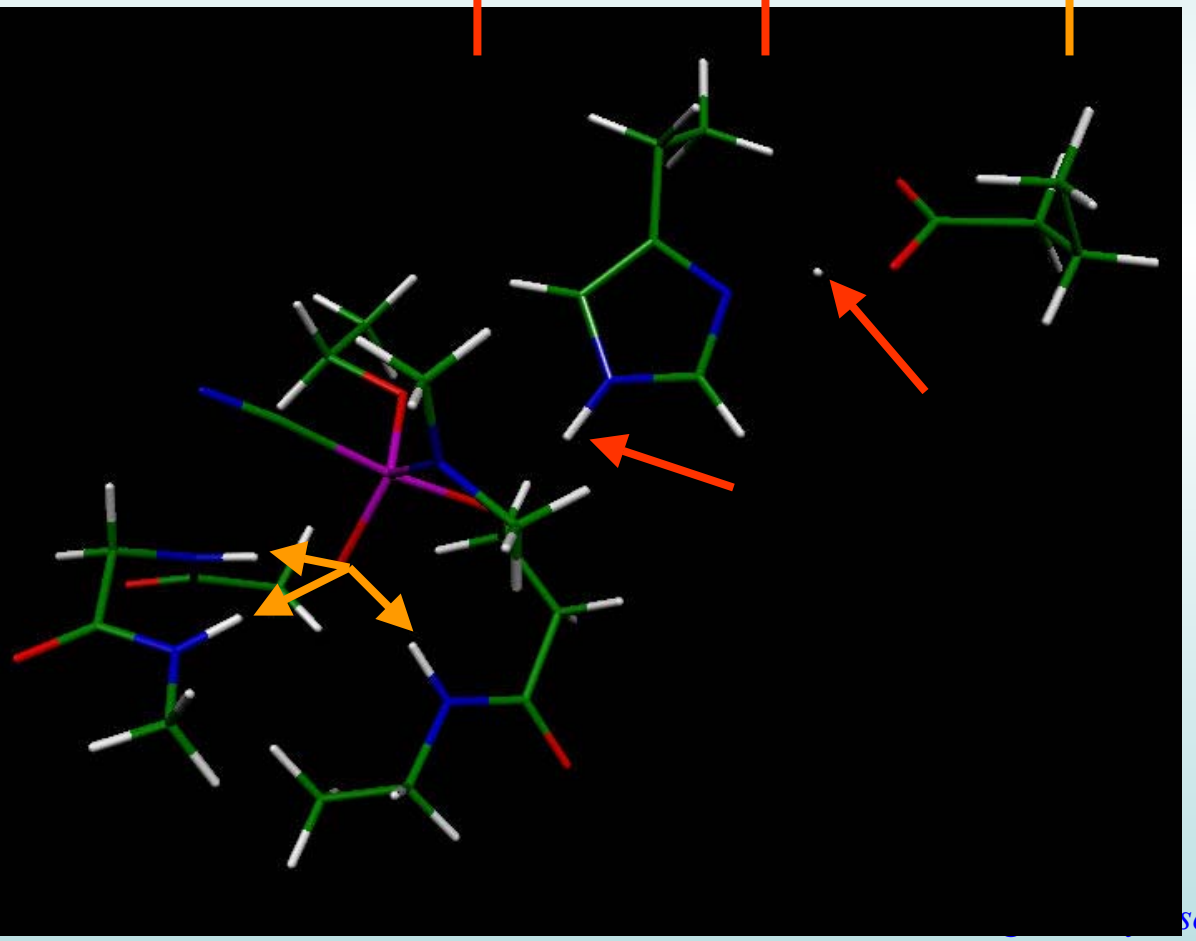
Pyridostigmine
b3lyp 631g(d,p)
Carbamylation of active site serine –
•oxyanion hole (somewhat)
• role of water again in LG migration
•importance of CHO H-bonds





SSHB in inhibited enzyme





Calculated proton NMR of bare enzyme b3lyp 6-31g(d,p) and determine shift WRT TSP b3lyp-61g(d,p)

SSHB?

- O-H-N <2.65Angstrom

□δ >15 ppm

Calculated Proton NMR shift

ACTOX

	H1 Shield	H1 dist	H1 angle	H2 Shield	H2 dist	H2 angle	H3 Shield	H3 dist	H4 Shield	H4 dist	H5 Shield	H5 dist
EXPT Massiah et al	14.4 (pH7.5)											
	17.7 (pH 5	2.62										
bare triad + OX	17.83	2.62	167.20	18.64	2.61	175.80	11.70	2.78	10.65	2.76	7.49	3.04
DFP	22.62	2.53	170.96	16.33	2.62	162.23	11.39	2.81	12.12	2.75	10.93	2.75
DFP w/o F-	18.60	2.61	170.20	11.42	2.79	151.46	8.57	2.87	8.55	3.01	7.86	3.00
DEFP	22.61	2.53	170.96	16.42	2.62	162.65	11.61	2.75	12.05	2.75	10.62	2.83
DMMP	23.36	2.51	171.76	17.72	2.59	164.48	11.99	2.72	12.72	2.73	11.53	2.77

SSHB *Sometimes* SSHB Normal HB

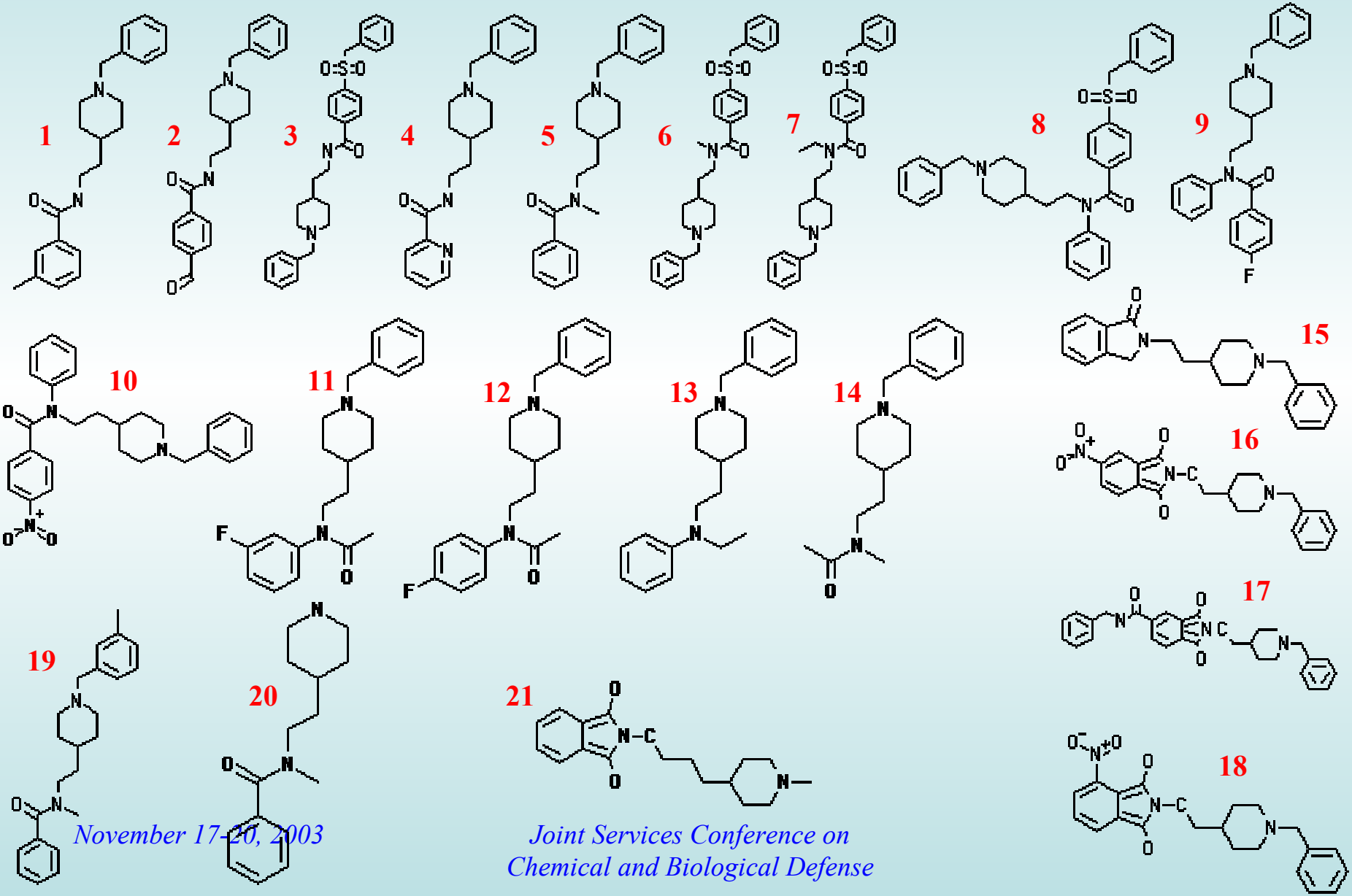
Calculated Proton NMR shift

ACTOX

	H1 Shield	H1 dist	H1 angle	H2 Shield	H2 dist	H2 angle	H3 Shield	H3 dist	H4 Shield	H4 dist	H5 Shield	H5 dist
bare triad + OX	17.83	2.62	167.20	18.64	2.61	175.80	11.70	2.78	10.65	2.76	7.49	3.04
S sarin + glu199	16.27	2.58	166.47	11.65	2.68	175.46	9.16	2.76	2.42	2.94	6.77	2.76
S sarin w/o F-	18.40	2.62	171.72	11.51	2.82	158.40	7.96	2.84	8.75	2.87	8.49	2.91
S sarin	22.34	2.53	170.97	17.12	2.61	172.17	9.54	2.83	12.31	2.75	11.37	2.78
S sarin aged	17.34	2.53	170.63	10.93	2.70	166.24	7.94	2.72	9.08	2.75	7.57	2.80
EXPT (Millard et al)		2.53										
S sarin oniom		2.63	164.64		2.86	137.42		2.53		2.49		2.50
R sarin	23.59	2.51	171.56	19.44	2.53	174.29	9.64	2.77	10.53	3.54	7.63	2.98

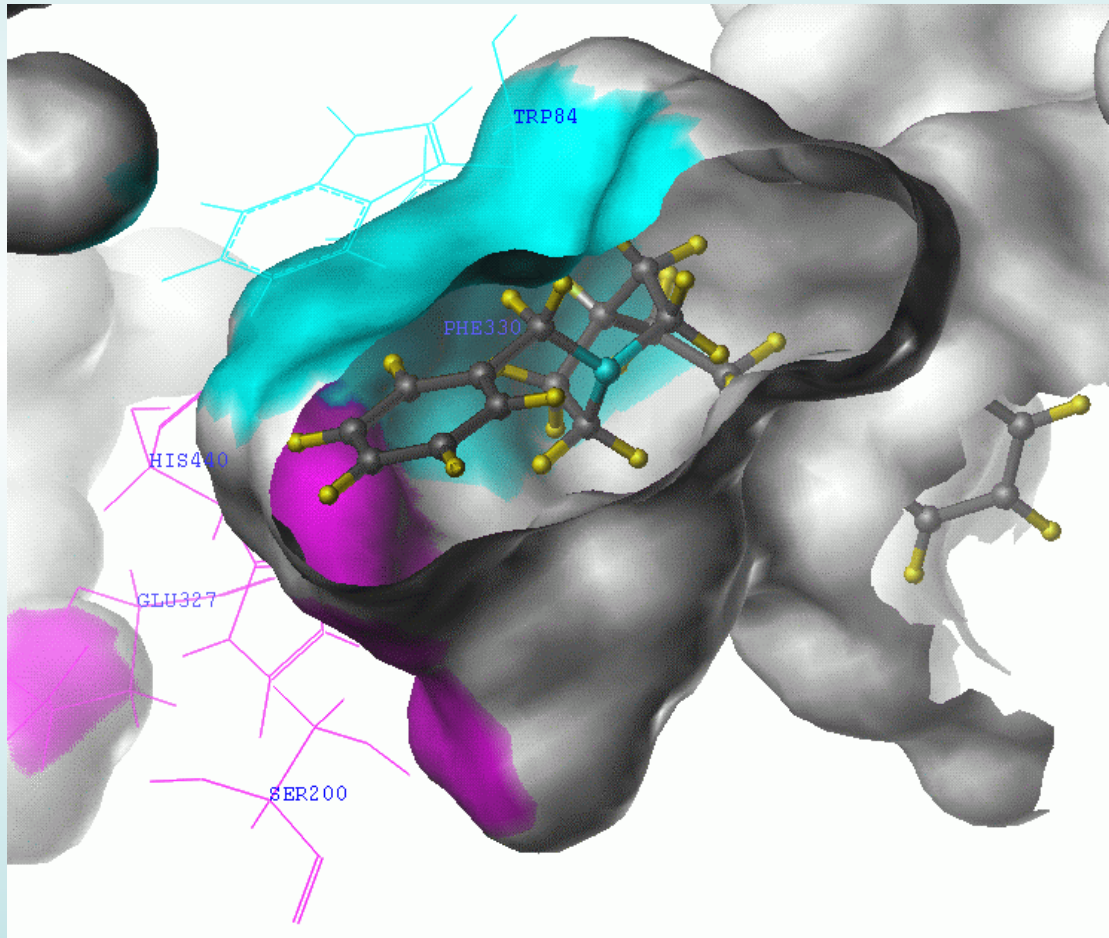
SSHB *Sometimes* SSHB Normal HB

Piperidinyl Analogs / Gorge Binders

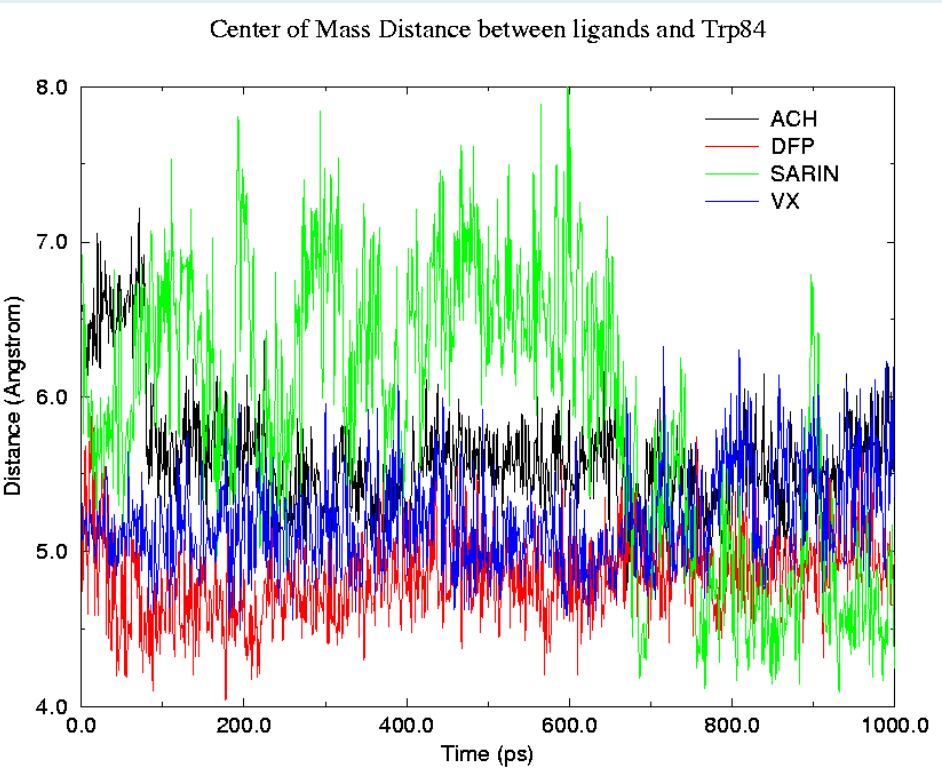


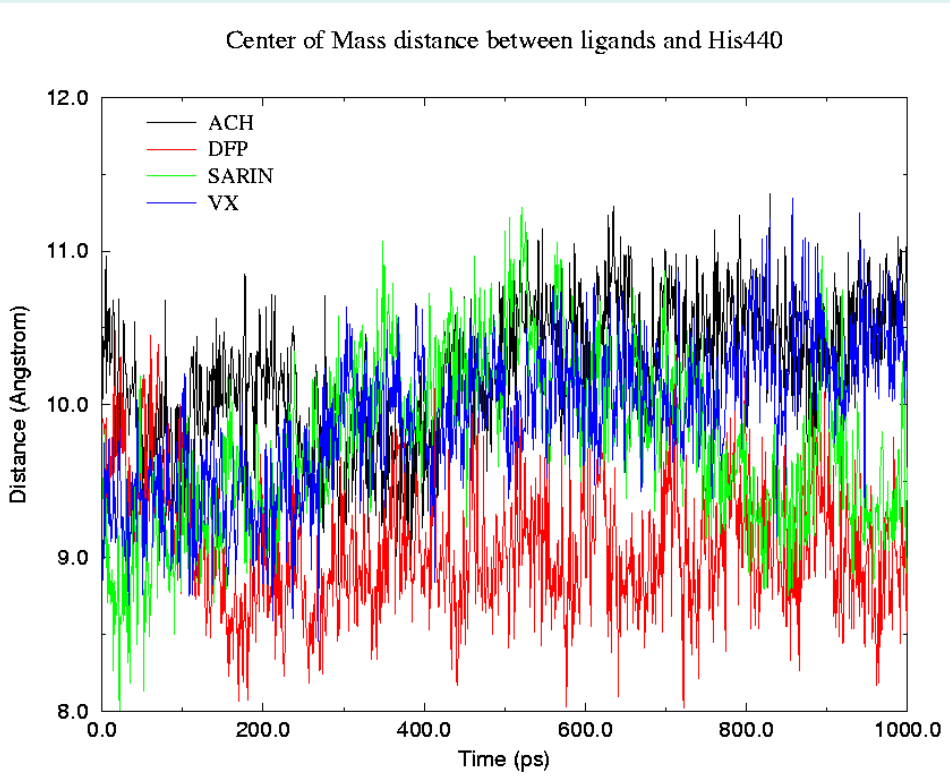
Gorge Binders: good correlation

	pIC50(nm)	D_Score
1	6.32790231704712	-141.139999389648
2	6.92081880569458	-153.020004272461
3	7.53760194778442	-169.710006713867
4	6.09690999984741	-130.779998779297
5	6.76955127716064	-141.320007324219
6	9.221848487854	-190.369995117188
7	9.52287864685059	-189.529998779297
8	9.221848487854	-196.570007324219
9	7.74472761154175	-171.0
10	8.26760578155518	-176.869995117188
11	7.18708658218384	-150.380004882812
12	6.68824625015259	-141.970001220703
13	4.92081880569458	-125.790000915527
14	6.18045616149902	-136.619995117188
15	7.00877380371094	-147.160003662109
16	7.90309000015259	-162.320007324219
17	8.65757751464844	-187.289993286133
18	8.04575729370117	-163.059997558594
19	6.83863210678101	-149.080001831055
20	4.58502674102783	-95.4199981689453
21	5.16749095916748	-102.610000610352

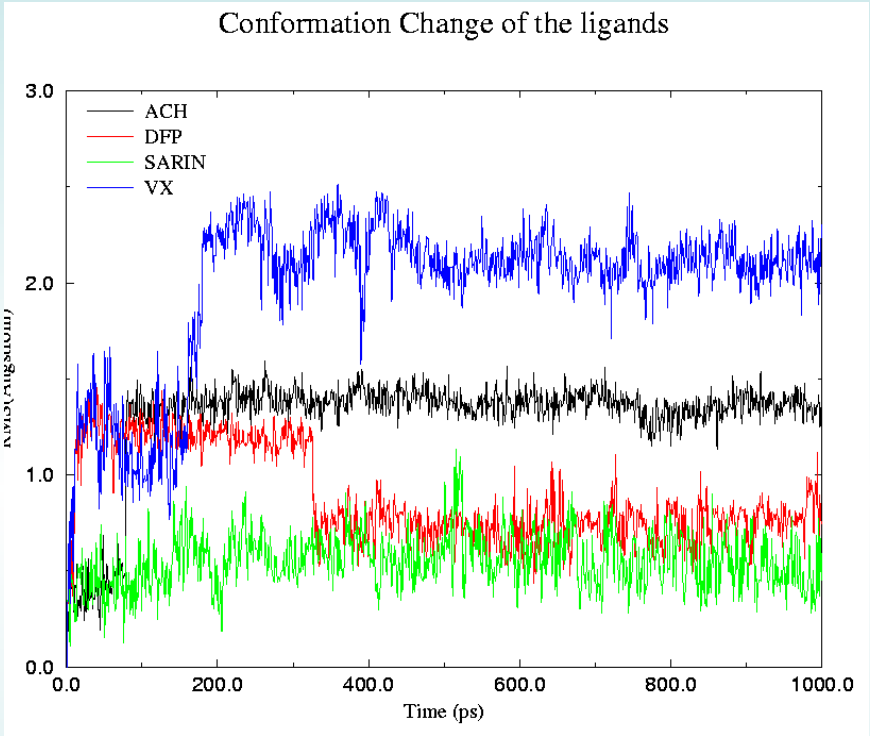


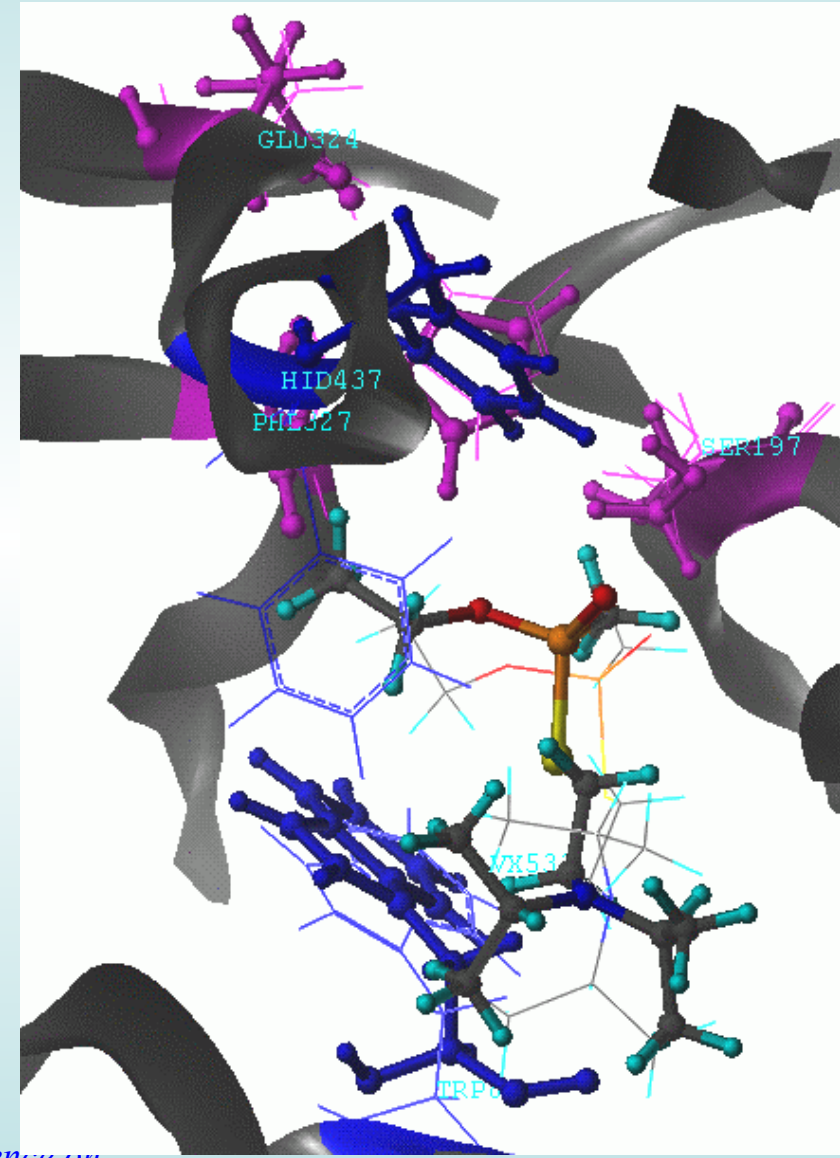
Gorge vs. Triad Binding



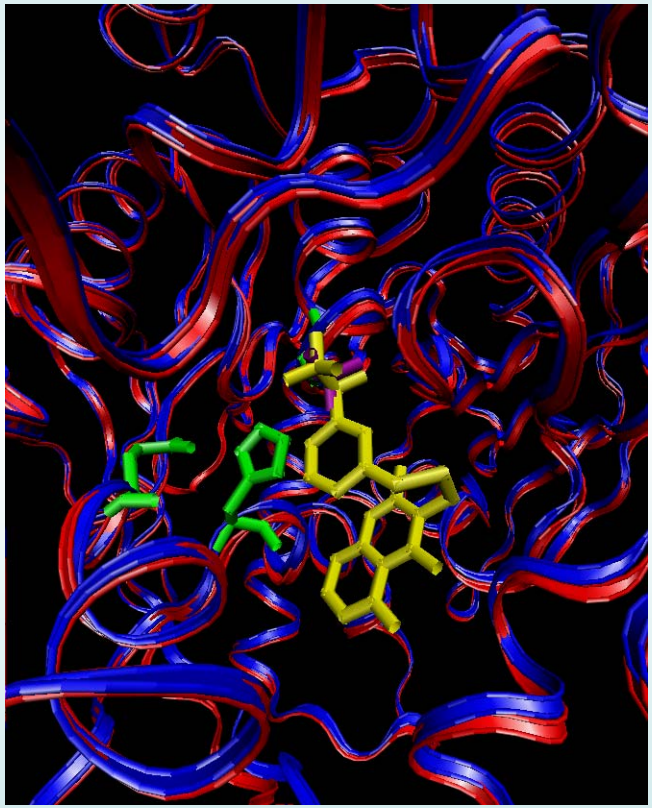


Gorge Admission





Conclusions/Future



Conclusions

- NMR confirmation of SSHB in agreement with expt
- Validated role of oxyanion hole
- Validated Role of solvent and leaving group migration
- Confirmed Stereoselectivity in model

Ongoing/Future:

- Aging and role of surrounding residues
- Mutagenesis
- Larger systems

Acknowledgements

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